observation that synaptic strength correlates with dendritic spine morphology leads to the hypothesis that the mushroom-like shape of dendritic spines functions as a receptor trap.
We developed a mimetic system to investigate dendritic spine morphology and its effects on receptor confinement and diffusion. Giant unilamellar vesicles (GUV's) are made from lipids using electroswelling. To mimic the mushroom-shaped morphologies of dendritic spines, a micromanipulator is used to pull membrane tubes from the GUV lipid bilayer. Trapping capabilities for different spine morphologies are assessed by tracking quantum dots attached to membrane lipids, thus mimicking receptors.
Results show a strong dependence of escape times on GUV morphology, as quantified by GUV radius and tube length. Instead of a trivial quadratic dependence of escape times on GUV radius we find a powerlaw dependence with an exponent of 2.85. This confirms the idea that receptors can be trapped by the morphology of a dendritic spine. Therefore the connection strength of a mushroom-shaped dendritic spine is much more stable than the strengths of stubby shaped dendritic spines.

Platform: Protein Structure and Conformation III

1724-Plat
Transmembrane Signaling through a Bacterial Heme Transporter
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Bacteria use diverse signaling pathways to control gene expression in response to external stimuli. In Gram-negative bacteria, the binding of some nutrients is sensed by their specific outer membrane transporter. A cascade of molecular interactions between several proteins, located in three subcellular compartments, is then used to send this signal from outside to inside the bacteria and upregulate the expression of genes related to the acquisition of these nutrients. We study a heme acquisition system (Has) developed by several commensal and pathogenic bacteria to acquire heme as iron source. Using multidisciplinary approach (NMR, Xray, SAXS and Electron Microscopy) we have determined the structure of multiprotein complexes involved in the Has signaling pathway. Furthermore, we have recently shown, for the first time, that a partially folded protein is involved in this process [1,2,3,4,5]. Our current data represent the first detailed characterization of this type of bacterial signaling.
Ref: 1:Krieg S et al 2009 PNAS; 2:Caillet-Saguy S et al JACS 2009; 4: Cardoso de Amorim et al PlosOne 2013; Malki et al PlosOne 2014.Wojtowicz et al, in preparation.

1725-Plat
Tom1 Modulates the Endosomal Function of Tollip via a Folding-Upon-Binding Mechanism
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Many cellular signaling processes require internalization of ubiquitylated cargo via endocytosis and early endosomes are the first sorting station for vesicular cargo. Adaptor protein complexes modulate signaling output in early endosomes by triggering cargo sorting for lysosomal degradation. Tollip, through its C2 domain, associates with endosomal membranes via phosphatidylinositol 3-phosphate (PtdIns(3)P) and recruits ubiquitylated cargo to these compartments via its C2 and CUE domains. Interestingly, binding of Tollip to PtdIns(3)P is inhibited by ubiquitin. Tom1, through its GAT domain, is recruited to endosomes by binding to cargo and the Tollip TBD region by an unknown mechanism. NMR data revealed that Tollip TBD is a natively unfolded domain that partially folds when bound to Tom1 GAT. Furthermore, the association of Tom1 to Tollip inhibits Tollip's binding to PtdIns(3)P. We hypothesize that Tom1 GAT binding to Tollip TBD causes conformational changes in Tollip that impairs its binding to PtdIns(3)P, increasing its commitment to protein sorting. Tollip TBD-Tom1 GAT forms a stable heterodimer, whose association is mainly driven by hydrophobic contacts of high affinity. Nuclear spin relaxation studies demonstrate that the N-terminal half structure of Tollip TBD, which contacts Tom1 GAT, is ordered, whereas the C-terminal half is highly unstructured. Ubiquitin binds to Tom1 GAT at a site that does not overlap with that for the Tollip TBD following a fast exchange regime. Cytosolic Tom1 is recruited to endosomal compartments when co-expressed with Tollip in mammalian cells, but mutations, which reduce 90,000-fold the association of these proteins, revert this effect. Accordingly, we propose that association of Tom1 to Tollip facilitate Tollip release from endosomal membranes, allowing Tollip to commit to endosomal cargo trafficking.
I respectfully request the Committee consider our work for oral presentation based on its interest to a broad audience.

1726-Plat
X-Ray Structure of a Calcium Activated TMEM16 Lipid Scramblase
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The TMEM16s or anoctamins constitute a class of eukaryotic membrane proteins that in mammals contain ten members with high sequence conservation. Despite their close relationship these proteins are characterized by a remarkable functional diversity. The family includes the long sought-after Ca^{2+}-activated chloride channels (TMEM16A and B) but also cation channels and lipid scramblases that support the exchange of lipids between the inner and outer leaflets of the bilayer in an ATP-independent manner. As part of the blood coagulation process, TMEM16F triggers the exposure of phosphatidylserine in blood platelets upon activation by Ca^{2+}. TMEM16C, D, G and J were suggested to work as scramblases as well, but with variable characteristics. Although we have by now gained considerable insight into the functional properties of certain family members, their architecture and the relation to mechanisms of action were so far unknown. Here we present the crystal structure of nhTMEM16, a fungal family member that operates as a Ca^{2+}-activated lipid scramblase. Each subunit of the homodimeric protein contains ten transmembrane helices and a hydrophilic membrane-traversing cavity that resembles a 'spiral staircase' and is exposed to the lipid bilayer as a potential site of catalysis. This cavity harbors a conserved Ca^{2+}-binding site located within the hydrophobic core of the membrane. Ca^{2+} binding by six residues, five of which carry a negative charge, controls the activation of scrambling in nhTMEM16. A triple mutant of residues in this site shows only weak scrambling activity that is no longer enhanced by Ca^{2+}.The structure thus reveals the general architecture of the family and its mode of Ca^{2+}-activation. It provides insights into potential scrambling mechanisms and will furthermore serve as a framework to unravel the conduction of ions in certain TMEM16 proteins.

1727-Plat
Autophagy: Solution Structure of the Atg17-Atg29-Atg31-Atg1-Atg13 Complex
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Autophagy is a complex bulk clearance mechanism that collects cellular material intended for degradation. In yeast, this highly conserved machinery comprises about 40 autophagy-related (Atg) proteins, most of which form multiprotein complexes. We elucidated the structure of the Atg17-Atg29-Atg31-Atg1-Atg13 complex in solution using small-angle X-ray scattering experiments (SAXS), coarse-grained simulations, and the ensemble refinement of SAXS (EROS) method. Our model consists of rigid domains based on the crystal structures of the Atg17-Atg29-Atg31 complex and of the Atg1-Atg13 complex. The modeling and simulation of disordered regions, which have not been resolved in the crystal structures, is crucial for the determination of the solution structures. Specifically, the Atg17 binding site on Atg13 has not been resolved and is located on such a disordered part of the protein. We therefore modeled disordered regions as flexible chains and link the Atg17 proteins and Atg13 proteins accordingly. We performed coarse-grained simulations for various multiprotein complex topologies and refined the resulting ensembles of structures using EROS. The resulting structural models account consistently for the SAXS measurements of the Atg17-Atg29-Atg31-Atg1-Atg13 supercomplex and several of its subcomplexes.

1728-Plat
Structural Studies of G-Alpha-Q Signaling
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The heterotrimeric G protein $G\alpha_q$ has been implicated in a variety of cardiovascular processes, such as blood pressure regulation. Structural studies of